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Distribution of Stenasellidae in Africa and description of a new species of *Metastenasellus* from Cameroonian groundwaters

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Abstract

During recent investigations of the groundwater fauna of Cameroon, specimens of a new species of the stygobitic genus *Metastenasellus*, *M. boutini* sp. nov. were collected in wells of the city of Douala. The new species can be easily distinguished from the other species of the genus by its relatively large size (up to 11 mm), pleonite 1 and 2 half the length of pereonite 7, the shape of pleopod 2 in males (presence of an external lobe on the protopodite, distal part of the spermatic duct slightly protruding out of the second article, lack of a distal seta on the exopodite), and uropod half the length of the pleotelson. Ecological data and a key to *Metastenasellus* species are provided. We also performed an exhaustive analysis of the literature on Stenasellidae in Africa to study the geographical distribution of the family in this continent and discuss some hypotheses about the origin of African species.

Keywords

biogeography, integrative taxonomy, Isopoda, stygofauna, tropical Africa

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Introduction

Over the last decades, investigations of groundwaters around the world have highlighted an unexpectedly high diversity of organisms forming the so-called stygobitic fauna. Stygobitic diversity even exceeds epigean diversity for some groups (Stoch 1995; Sket 1999; Gibert and Culver 2009). This is particularly the case for freshwater crustaceans; for instance, 38% of known freshwater species in Europe live only in groundwaters (Sket 1999). Despite important progress in our knowledge of groundwater biodiversity, data from the different parts of the world are still heterogeneous, with very poorly known regions (Gibert and Culver 2009). This is particularly true for continental Africa, where data about the diversity and distribution of the stygobites are scarce (Tuekam Kayo et al. 2012) and mainly reported in a few taxonomic papers.

Among the stygobitic species living in Africa, the order Isopoda, with at least 80 species, represents around 30% of the total number of groundwater species of the continent (Tuekam Kayo et al. 2012). African subterranean Isopod species belong to 7 families (Asellidae, Cirolanidae, Lepidocharontidae, Microcerberidae, Microparasellidae, Protojaniridae, and Stenasellidae). Stenasellidae harbors the highest number of species, with 23 currently known species distributed into 6 genera.

In this context, the goal of our study was to complete existing knowledge on the diversity and distribution of stenasellid isopods in Africa. We performed extensive sampling campaigns in Central Africa, in particular in Cameroon, starting in 2010. These campaigns revealed the presence of a new species, *Metastenasellus camerounensis* Zebaze Togouet, Boulanouar, Njiné & Boutin 2013 – but also of several other new species of *Metastenasellus* in Cameroon (Tuekam Kayo et al. 2012; Nana Nkemegni et al. 2015) and Benin (Eme et al. 2018) that remained undescribed. During surveys in southwestern Cameroon, a new species of *Metastenasellus* was found in wells near Douala. In this study, we describe this new species using morphological and molecular techniques and its ecology, we propose an identification key to all species of the genus *Metastenasellus*, and we summarize the published literature on the distribution of Stenasellidae in Africa.

Materials and methods

Study sites and faunal sampling

Between 2019 and 2021, 5 sampling campaigns (3 during the rainy season and 2 during the dry season) were carried out in 41 wells in the city of Douala. The wells were located in alluvial sediments near the Atlantic coast (maximum distance from the ocean 16 km) with an elevation lower than 65 m a.s.l., and a maximum depth of 9 m. The city of Douala has warm and humid climate conditions, with an average annual temperature of 27.0 °C and an average humidity level of 83%, with around 4,000 mm of precipitation *per* year (Olivry 1986). The dry season extends from December to February and a long rainy season extends from March to November.

Faunal samples were collected from the bottom of the wells using a modified phreatic net sampler (30 cm diameter aperture and 64 µm mesh size: Cvetkov 1968).

Water samples were collected in each well at each sampling date. Temperature, pH, electrical conductivity, and dissolved oxygen were measured directly in the field with a mercury thermometer, a portable pH-meter (CG 818, Schott instruments), and a portable conductivity meter and optical dissolved oxygen meter (HQ30d, Hach Lange), respectively. . The water was transported directly to the laboratory using polyethylene bottles under cool storage for additional analyses: alkalinity, dissolved carbon dioxide, calcium, magnesium, chemical (CDO) and biological (BDO5) oxygen demand, turbidity, and nutrient contents. The differences in physico-chemical parameters of water in wells with or without the new species were assessed using non-parametric Mann-Whitney U Tests using the STATISTICA v12 (StatSoft) software.

Morphological study

The specimens were dissected and mounted on microscopic slides in Faure's mounting medium, after maceration +in lactic acid and staining with pink lignin. Body parts were digitally drawn using a Wacom tablet and the Adobe Illustrator software package (Adobe).

Molecular analysis

Total genomic DNA was extracted from a part of an animal using NucleoSpin Tissue Kits (Machery-Nagel) following the manufacturer's instructions (Düren, Germany). A fragment of the mitochondrial COI gene was amplified using LCO1490/HCO2198 (Folmer et al. 1994) and UCOIR/UCOIF (Costa et al. 2009) primers. Touchdown Polymerase chain reactions (TD PCRs) (Korbie and Mattick 2008) were performed in a final volume of 27 µl containing 12 µl of water, 10.5 µl of Type-it PCR Master Mix (Qiagen, Germany), and 1.8 µl of each primer (5 µM). A denaturation step at 95 °C for 5 minutes was followed by 35 cycles (30 seconds at 95 °C, 90 seconds at each temperature and 30 seconds at 72 °C), with a final extension step of 30 minutes at 70 °C. TD-PCRs are characterized by an initial annealing temperature (55 °C in our study) above the projected melting temperature (Tm) of the primers, and then progressive transitions to a lower, more permissive annealing temperature over the course of the successive cycles (-0.5 °C per cycle during the first 8 cycles). The next 27 cycles were performed at 51 °C.

The PCR results were checked by gel electrophoresis, then the PCR products were purified and sequenced in both directions by the Eurofins sequencing facility or with an Applied Biosystem 3130 XL sequencer in the DNA sequencing facility of the Institute of Genetics and Development of the University of Rennes (https://igdr.univ-rennes1.fr/en). All COI sequences obtained in four wells are deposited on Genbank (assession numbers OL514108; OL514109; OL514110; OL51411 and OL51412) and on the Barcode of Life Data systems (BOLD) (BOLD process id: METAF001-21, METAF002-21, METAF003-21, METAF004-21 and METAF005-21).

The new COI sequences were supplemented by COI sequences downloaded from Genbank: two sequences of *Metastenasellus* species from Benin (accession numbers KY623773.1 and KY623774.1); four from *M. camerounensis* from Cameroon (accession numbers KY623769.1; KY623770.1; KY623771.1; KY623772.1), and two from an unknown *Metastenasellus* species from Cameroon (accession numbers KY623775.1; KY623776.1). All sequences were aligned with the MUSCLE algorithm (Edgar 2004) implemented in SEAVIEW ver. 5 (Gouy et al. 2021)to explore the *Metastenasellus* species diversity using different species delimitation methods implemented in iTAXOTOOLS 0.1 (Vences et al. 2021). Firstly, according to distance-based methodologies: the Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al. 2021) and the distance-based Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012). Secondly, the results of the genetic distance-based species delimitation were cross-validated with the phylogenetic tree-based delimitation methods, namely: Generalized Mixed Yule Coalescent (GMYC) (Pons et al. 2006) and multi-rate Poisson tree processes (mPTP) (Kapli et al. 2017). For tree-based species delimitation methods, we built a phylogenetic tree under a maximum likelihood framework (ML) using PhyML v3.1 algorithm (Guindon et al. 2010) implemented in SEAVIEW ver. 5 (Gouy et al. 2021). For the tree, we used HKY85+I model, selected as the best-fit model of evolution with jModelTest 2 (Darriba et al. 2012).

For final visualization with already sequenced species of *Metastenasellus*, the neighbor-joining tree of all COI sequences, using the Kimura two-parameter (K2P) model of evolution (Kimura 1980) with 1000 bootstrap replicates, was created in SEAVIEW ver. 5 (Gouy et al. 2021).

Distribution of Stenasellidae in Africa

An exhaustive survey of the literature on Stenasellidae (species descriptions, identification keys, monography, PhD theses, books, papers) was carried out for all the species known in continental Africa. All maps were drawn with Arcgis Desktop 10.4 software (Esri) and using GIS data available on DIVA website (http://www.diva-gis.org) in WGS 84 datum.

Results

Species description

Order Isopoda Latreille, 1817
Suborder Asellota Latreille, 1802
Superfamily Aselloidea Latreille, 1802
Family Stenasellidae Dudich, 1924
Genus Metastenasellus Magniez, 1966

***Metastenasellus boutini* Pountougnigni, Piscart & Zebaze Togouet, sp. nov.**
http://zoobank.org/B56F7FB4-8AE2-4B9A-8B26-8F4FDDF0E327

Diagnosis. *Metastenasellus boutini* n.sp. is characterized by pleonites 1 and 2 around 50% as long as pereonite 7, the presence of sternal spine on each dactylus of pereopods 2–7 and endopodite of pleopod 2 in males long and large with a helicoidal spermatic duct.

Material examined. Type-specimen: *Holotype* ♂ (9.8 mm), mounted on 2 slides and deposited at the Muséum national d'Histoire Naturelle de Paris (MNHN, France) under voucher number MNHN IU-2021-1818.

Type-locality. Cameroon, in PK21 quarter, Douala city, 04°07'16"N; 09°49'41.4"E, in a well at 60 m a.s.l. and 8 m depth, 24 April 2021. ***Paratypes***: 5 ♂♂ in vials; same data as for holotype; MNHN IU-2021-1819 • 3 ♀♀ in vials; same data as for holotype; MNHN IU-2021-1820.

Other material examined. 22 specimens collected in seven wells around the type locality (Table 1).

Etymology. The epithet *boutini* refers to the name of Dr. Claude Boutin who initiated many studies on stygofauna in northern and central Africa.

Description of male. *M. boutini* sp. nov is a relatively medium-sized stenasellid, length up to 11 mm in males. Cephalon short and rounded with a concave rostral margin and convex distally. Pereonites 1 to 7 well developed, the 6th and 7th being the longest. Pleonites 1 and 2 free and as long as 50% of the length of the pereonite 7. Pleotelson subrectangular with a pointed caudal margin and partially covering the protopodite of the uropod.

Antenna 1 (Fig. 1B) slightly longer than peduncle of antenna 2, flagellum with 12–15 articles, the last 6 bearing a single distal aesthetasc (*lamina olfactoria*), the first two articles of the peduncle are longer than the others and bear one and two sensory plumose setae, respectively. Antenna 2 (Fig. 1C) around 30% of body length, 2.5 times as long as antenna 1, flagellum composed of a variable number of short articles (32 to 48 articles for specimens between 9 and 10 mm), peduncle articles 5 and 6 bearing one and two sensory setae, respectively; exopodite vestigial (*squama*) and scale-like on posterior margin of article 3 with a spine and a long simple seta on the apex. Mandibles asymmetrical, incisor processes with four teeth; left mandible (Fig. 1D) with a well-developed *lacinia mobilis* with four teeth, followed by a row of 18 serrate setae and 12 toothed setae; right mandible (Fig. 1E) with a shorter four-toothed *lacinia mobilis* followed by a row of 18 serrate setae and 12 toothed setae; palp tri-articulated, the first article with one long simple distal seta, the second with two simple setae and ten single sided serrated setae, the last article has 12 single sided serrated setae, the two terminal ones being longer. Maxilla 1 (Fig. 1F) endite clearly separated from exite with two groups of ciliated setae at the apex separated by one simple seta and four setules on external margin; exite with 12 apical serrated setae with one to six teeth and one ciliated seta. Maxilla 2 sympod (Fig. 1G) bearing on its medial margin 7 simple setae and one strong seta; endite with 14 ciliated setae at the apex, middle lobe with 7 ciliated setae and external exite with 5

Table 1. Location of wells sampled in the quarter PK21 of Douala.

Wells	Latitude, Longitude	*M. boutini*
P1	4.124111, 9.826861	Yes
P2	4.124083, 9.827056	No
P3	4.121444, 9.828333	Yes
P4	4.121111, 9.828167	Yes
P5	4.120694, 9.827278	Yes
P6	4.120639, 9.826028	No
P7	4.119528, 9.826389	Yes
P8	4.119056, 9.826528	No
P9	4.118778, 9.827111	Yes
P10	4.119083, 9.825917	Yes

ciliated setae. Maxilliped (Fig. 1H) endite bearing 4 plumose setae, 3 serrated setae, and a pair of coupling hooks on medial margin; palp 5-articulated, articles 2 and 3 distinctly longer and stronger than the other three, articles 1–5 bearing from base to apex 4, 9, 14, 14 and 2 simple setae on medial margin, article 4 with one additional simple seta on its external margin, article 5 with 10 apical simple setae.

Gnathopod (pereopod 1) (Fig. 2A) short, powerful, and haptorial with a dense chaetotaxis and strongly armed on the ventral margin of the last four articles; basis with 8 short setae on the ventral margin; ischium subtrapezoidal and bearing two setae on each margin; merus and carpus subtriangular, bearing on the ventral margin 5 and 6 long setae, 2 and 7 pen-like setae, respectively, merus bearing 2 additional long and strong setae on its outer tip; propodus enlarged with simple setae alternating with 9 toothed setae and 4 strong proximal denticulated setae; dactylus armed with 7 toothed setae and few simple setae on its ventral margin and 8–10 simple setae on the dorsal margin.

Pereopods 2 to 7 (Fig. 2B-D; Fig. 3) typically ambulatory, slender, and rather long, with a more or less developed chaetotaxis and a similar morphology with sensorial setae on dorsal margin of the basis, a strong armature of all articles with spines of various sizes and few setae, one strong sternal spine on each dactylus; pereopod 2 (Fig. 2B) and 6 (Fig. 3B) also bearing a sensorial seta on the distal part of dorsal margin of the propodus and carpus, respectively.

Pleopod 1 (Fig. 4A) uniramous; propodus subrectangular and convex on its external margin, glabrous and without retinacula; exopodite oval, 2.5 times as long as wide with 17 distal setae, the 5 medial ones being the longest. Pleopod 2 (Fig. 4B) biramous, rami clearly separated; protopodite subpentagonal with an oblique distal part and a developed process on the external margin, slightly overreaching the first segment of the exopodite; endopodite biarticulated, proximal article not clearly delimited and ankylosed to the second one, second article highly developed, fusiform and twisted, containing a helicoidal spermatic channel with a large proximal-internal afferent opening and a smaller distal efferent orifice, surrounded by chitinous teeth; exopodite, narrower than endopodite, biarticulate with a rounded second article, larger than the first article with one subterminal seta and 2 or 3 marginal external

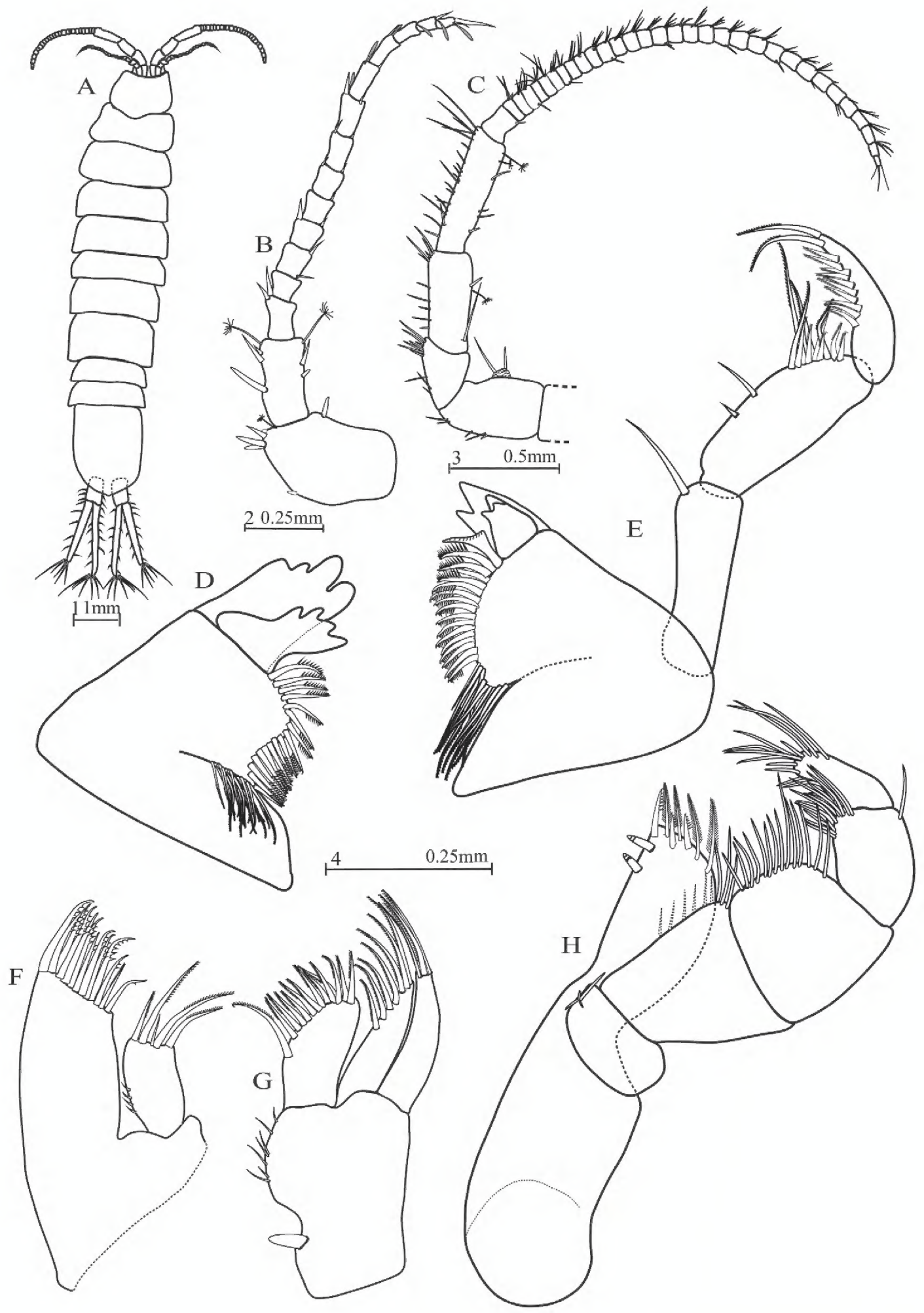

Figure 1. *Metastenasellus boutini* sp. nov., ((**A–H**), ♂ holotype 9.8 mm) (**A**) habitus (scale 1) (**B**) antenna 1 (scale 2) (**C**) antenna 2 (scale 3) (**D**) left mandible (scale 4) (**E**) right mandible (scale 4) (**F**) maxilla 1 (scale 4) (**G**) maxilla 2 (scale 4) (**H**) maxilliped (scale 4).

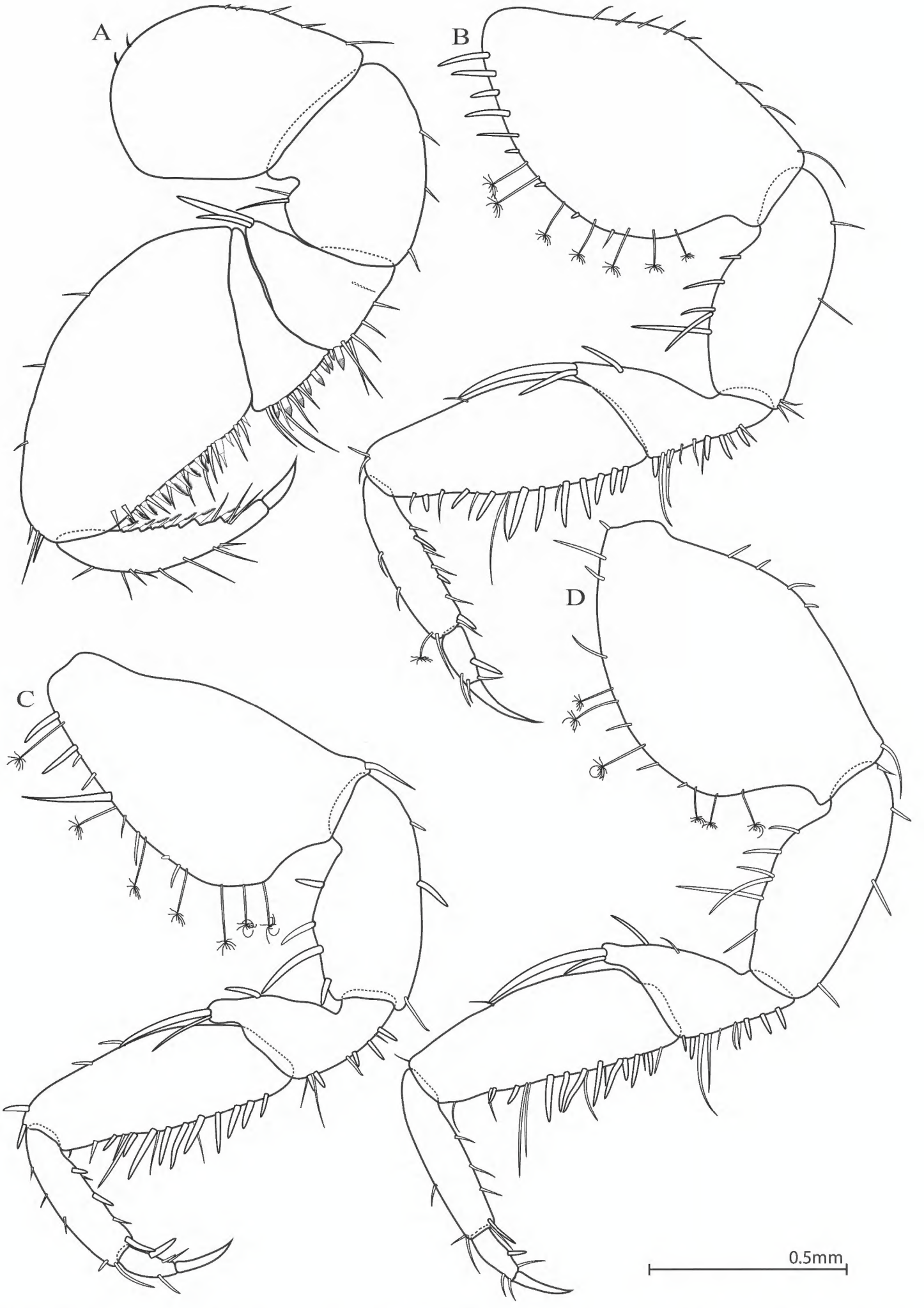

Figure 2. *Metastenasellus boutini* sp. nov., ((**A–D**) ♂ holotype 9.8 mm) (**A**) pereopod 1 (**B**) pereopod 2 (**C**) pereopod 3 (**D**) pereopod 4.

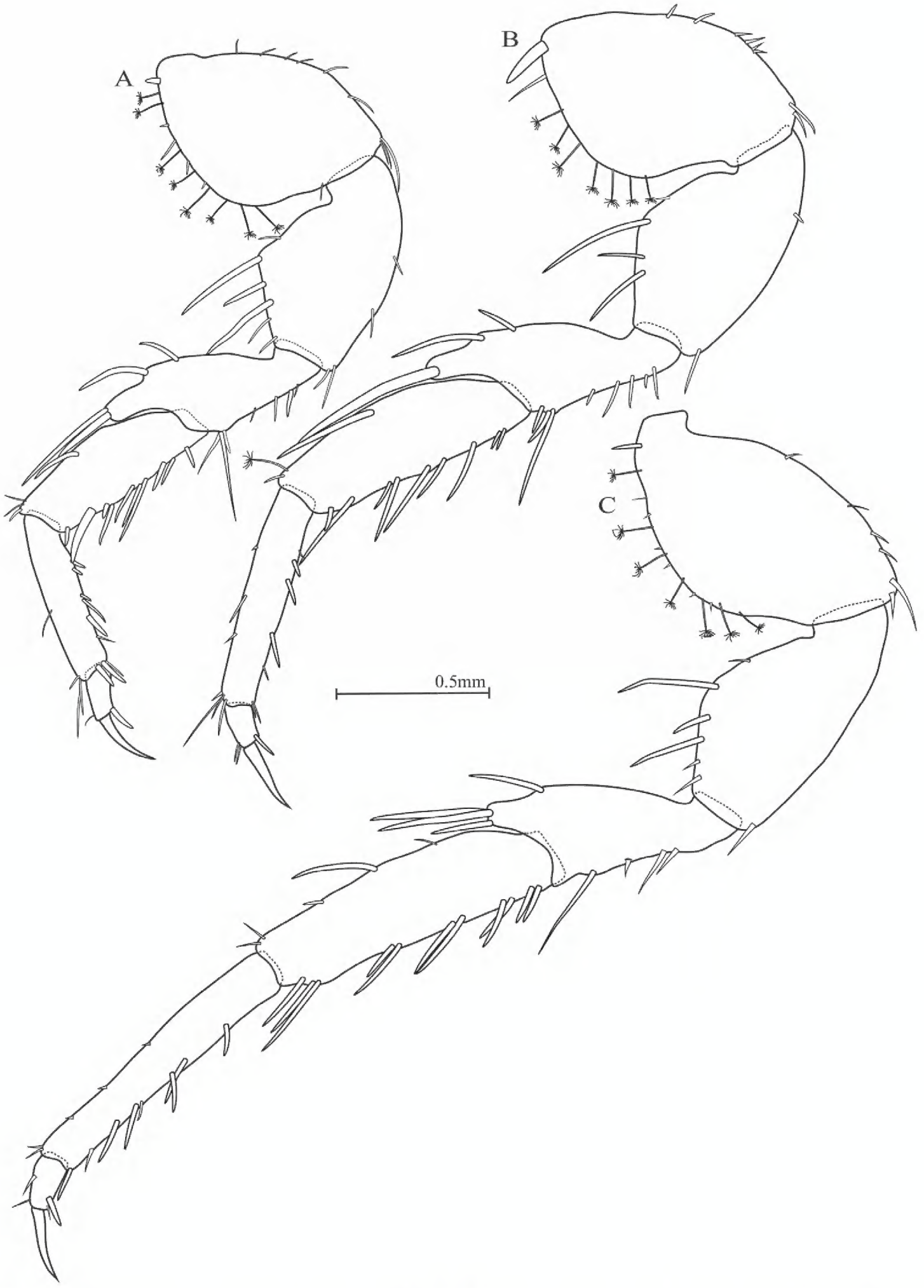

Figure 3. *Metastenasellus boutini* sp. nov., ((**A–C**) ♂ holotype 9.8 mm) (**A**), pereopod 5 (**B**) pereopod 6 (**C**) pereopod 7.

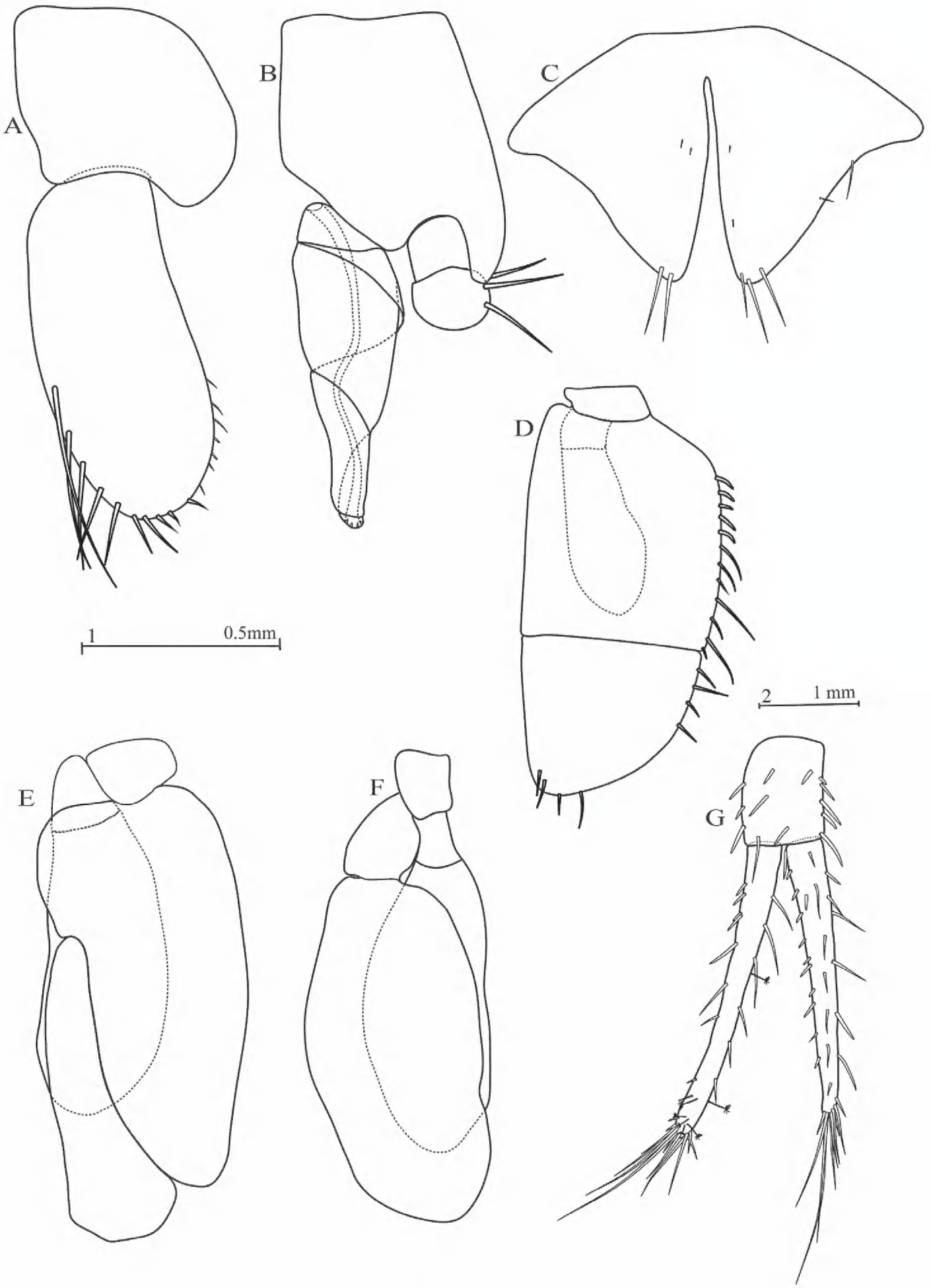

Figure 4. *Metastenasellus boutini* sp. nov., ((**A–B, D–G**) ♂ holotype 9.8 mm (**C**) ♀ paratype 8 mm) (**A**) pleopod 1 (scale 1) (**B**) pleopod 2 (scale 1) (**C**) ♀ pleopod 2 (scale 1) (**D**) pleopod 3 (scale 2) (**E**) pleopod 4 (scale 1) (**F**) pleopod 5 (scale 1) (**G**), uropod (scale 2).

setae. Pleopod 3 (Fig. 4D) with a very short protopodite; endopodite smaller than the first article of the exopodite and biarticulated, with the second article, larger, distally oval; exopodite also biarticulated, first article long and subrectangular, bearing on its external margin 12 setae of varying size; second article subtriangular, shorter than the first one and bearing 4 simple proximal setae on its external margin and 4 apical setae. Pleopod 4 (Fig. 4E) with a short protopodite; endopodite biarticulated with the second article more than 3 times longer than the first one; exopodite large, glabrous, with very oblique interarticular suture, first article much larger and longer than the second one. Pleopod 5 (Fig. 4F) with a subrectangular protopodite; endopodite biarticulated with the second article more than 4 times as long as the first one; exopodite biarticulated, slightly longer than endopodite; first article short, second article much larger and longer than the first one. Uropod (Fig. 4G) biramous as long as the pleotelson; protopodite subrectangular with dorsal and marginal setae; endopodite slightly longer than exopodite, both with numerous setae and spines and with several long apical setae; sensorial setae present only on the endopodite.

Female. Females are very similar to males with a reduced chaetotaxis on uropods. They are slightly longer than males, size up to 12 mm. Pleopod 2 (Fig. 4C) typical of female *Metastenasellus* formed by two single sub-triangular plates, with external and distal angles rounded. The lateral and apical margins of plates varied from concave to convex and bear few setae (0 to 3) each. Left and right pleopodal plates joined over 10% of their proximal part and are well separated on their distal part. Each plate bearing several (5–10) small ventral setae.

Differential diagnosis

The new species differs from most of species of *Metastenasellus* by the presence of an external lobe on the protopodite of pleopod 2 which is known only in *Metastenasellus leysi* Magniez, 1985. However, *M. boutini* differs from *M. leysi* by many other characteristics such as the total length (< 3.5 mm for *M. leysi*); the number of articles on flagellum of antenna 1 and 2 (2 and 13, respectively) much reduced for *M. leysi* in comparison with *M. boutini* (15 and 48, respectively); pleonites 1 and 2 as long as pereonite 7 for *M. leysi* (as long as 0.5 fold pereonite 7 for *M. boutini*). The shape of the endopodite of pleopod 2 of *M. boutini* is also characterized by a distal part of spermatic duct slightly protruding out the second article. By these characteristics, the new species strongly differs from *M. camerounensis*, *M. leleupi* (Chappuis 1951), *M. dartivellei* (Chappuis 1952), *M. congolensis* (Chappuis 1951), and *M. powelli* Magniez 1979. By its spermatic duct, *M. boutini* resembles to *M. wikkiensis* Lincoln 1972 and *M. tarrissei* Magniez 1979. It differs from *M. wikkiensis* by a shorter size of the uropod, as long as 50% the pleotelson for *M. boutini* (as long as 2 fold the length of the pleotelson in longest specimens of *M. wikkiensis*) and by the absence of the terminal setae on the second article of the exopodite of pleopod 2 (terminal setae present in *M. wikkiensis*). *M. boutini* differs from *M. tarrissei* by the total length and the number of article on flagellum of antenna 1 (8 for *M. tarrissei* and 15 *M. boutini*); pleonites 1 and 2 shorter than pereonite 7 for *M. boutini* (as long as the length of pereonite 7 for *M. tarrisei*).

Molecular analysis

We sequenced and analyzed DNA from five individuals from four wells, including the type locality at Douala. Based on the Folmer's fragment of COI marker, the new species is clearly distinct from the other species sequenced in Cameroon and in Benin. The pairwise genetic distances between *M. boutini* and all other species varied between 22.4 and 27.8% for Cameroonian species and even 28.2% for the species in Benin (Fig. 5). In addition to genetic distance and morphological distinctness, all delimitation methods clearly highlighted the existence of at least five distinct lineages of *Metastenasellus* in the material available for Africa (Fig. 5). All delimitation methods confirmed that individuals from wells at PK21 belong to the same lineage. Consequently and in addition to morphological distinctness, molecular data support the hypothesis that *M. boutini* can be considered as a new species, which strongly differs from *M. camerounensis* and the two other species already sequenced in Cameroon indicated as *Metastenasellus* sp1 and sp2.

Ecology and distribution

The new species *M. boutini* was collected in 7 of the 10 wells sampled in quarter PK21 of Douala (Table 1) city but was not found in the other 31 wells sampled in the same city. Isopod abundance in the wells was two-fold higher during the dry season than in the wet season.

The water chemistry of phreatic water in this part of the city was very acidic (pH = 4.2 ± 0.4), relatively warm, and well oxygenated with relatively low concentrations of calcium and magnesium (Table 2). We did not observe any statistically significant difference between the wells that harbored isopods and the wells without isopods (p-values > 0.092), whether in terms of physical traits (total depth, water depth) or physico-chemical parameters (Table 2). We also compared the seasonal variation of the stability of environmental conditions of the wells that harbored isopod *vs.* the wells without isopods, but no difference was observed (data not shown).

Distribution of Stenasellidae in Africa

The known distribution of Stenasellidae in Africa is very patchy (most of the species are known only from their type localities). Genera belong to three main geographic groups (Fig. 7). One group located in the eastern part of the continent (Kenya and Somalia) include two genera and eight species: *Acanthastenasellus forficuloides* Chelazzi & Messana, 1985; *Stenasellus agiuranicus* Chelazzi & Messana, 1987; *S. costai* Lanza, Chelazzi & Messana, 1970 ; *S. kenyensis* Magniez, 1975; *S. migiurtinicus* Messana, Chelazzi & Lanza, 1974; *S. pardii* Lanza, 1966; *S. ruffoi* Messana, 1993; *S. simonsi* Messana, 1999. A second group is located in north-western Africa and includes three genera and 10 species. Most of them are located in western Africa: *Parastenasellus chappuisi* (Remy, 1938); *Magniezia africana* (Monod, 1945); *M. gardei* Magniez, 1978; *M. guinensis* (Braga, 1950), *M. laticarpa* (Birstein, 1972); *M. studiosorum* Sket, 1969.

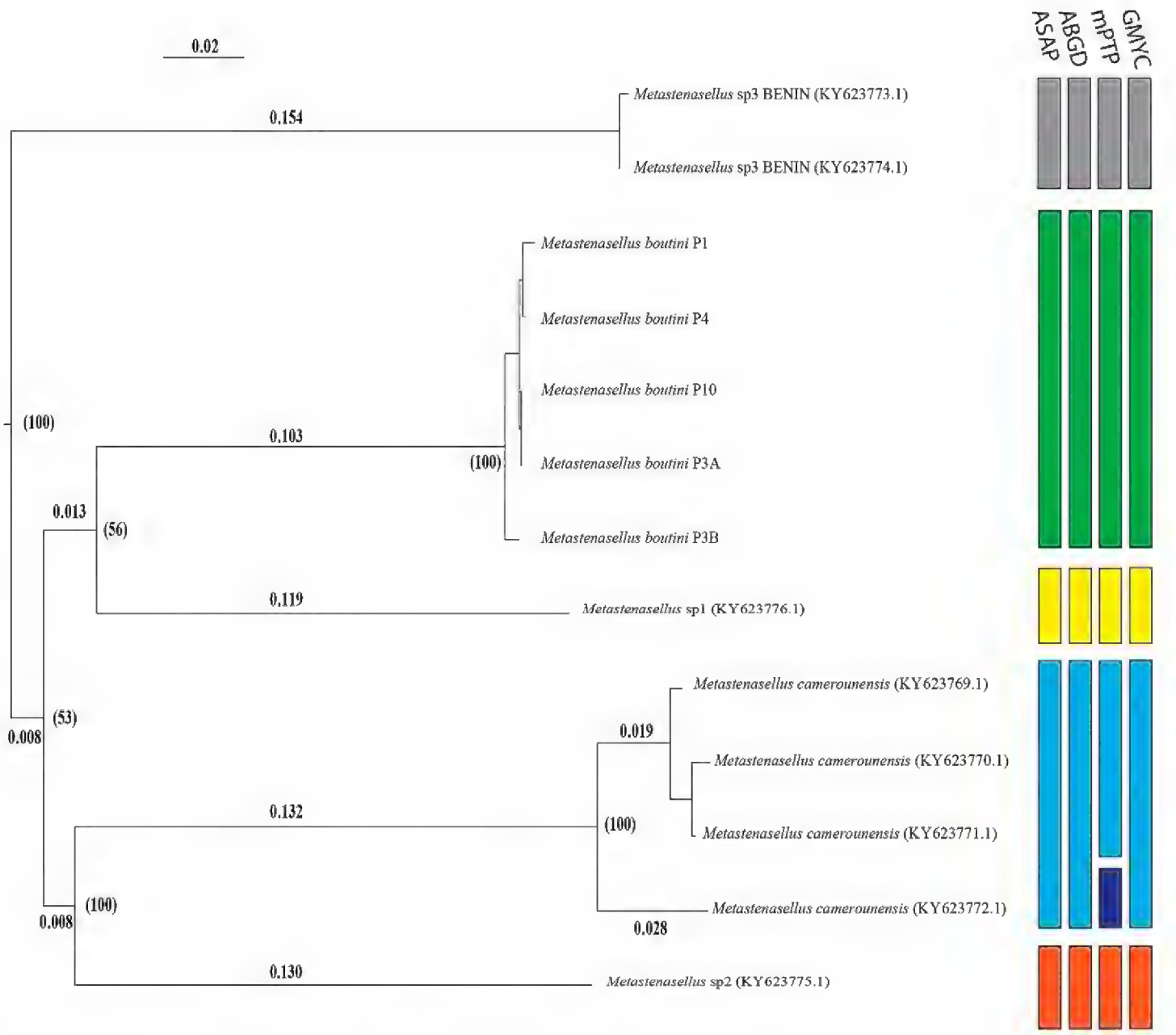

Figure 5. Neighbor-joining tree of the identified COI gene haplotypes. The evolutionary distances were computed using the Kimura two-parameter (K2P) model. Numbers between brackets in front of the nodes indicate bootstrap support (1,000 replicates). Boxes on the right indicate the best partition of species using ASAP, ABGD, mPTP and GMYC delimitation methods.

Table 2. Mean (min–max) values of physico-chemical parameters of wells sampled in quarter PK21 where *M. boutini* were found at Douala.

	Wells with *M. boutini*	**Wells without isopod**
Depth (m)	7.4 (6 – 9)	7.8 (7.6 – 8)
Water layer (cm)	157.7 (30 – 310)	116.2 (30 – 245)
Temperature (°C)	26.2 (22.4 – 29.5)	26.2 (22.7 – 28.7)
pH (IU)	4.2 (3.4 – 5.1)	4.23 (3.92 – 4.92)
O_2 (mg/L)	5.3 (3.8 – 8.2)	5.31 (3.3 – 7.4)
Electrical Conductivity (µS/Cm)	587 (155 – 822)	646 (192 – 947)
$N0_3^-$ (mg/L)	1.3 (0 – 3.5)	1.0 (0 – 3.1)
PO_4^{3-} (mg/L)	0.95 (0 – 3.1)	0.8 (0 – 2.9)
Ca^{2+} (mg/L)	2.0 (0.2 – 4)	2.1 (1.1 – 2.6)
Mg^{2+} (mg/L)	4.4 (**0.**3 – 11.4)	8.3 (1.3 – 14)

Only one species (*Johannella purpurea* Monod, 1924) of the genus *Johanella* is present in Algeria. The third group is only composed by the genus *Metastenasellus* and is widely distributed from the Democratic Republic of Congo to Algeria with 9 known species.

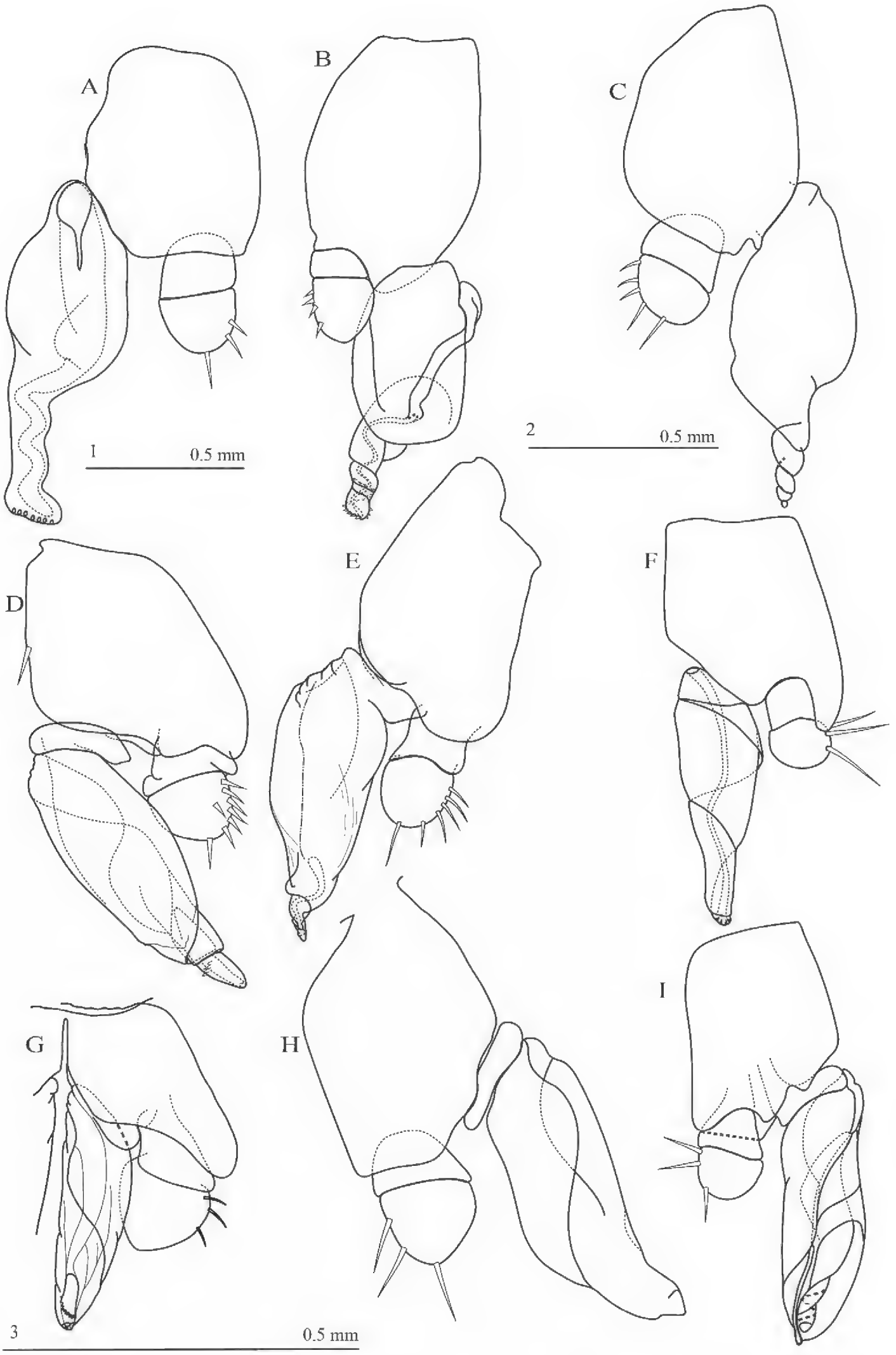

Figure 6. Pleopods 2 of males *Metastenasellus* as drawn in original descriptions (**A**) *M. leleupi* (scale 1) (**B**) *M. camerounensis* (scale 1) (**C**) *M. dartivellei* (scale 2) (**D**) *M. powelli* (scale 3) (**E**) *M. congolensis* (scale 1) (**F**) *M. boutini* (scale 2) (**G**) *M. leysi* (scale 3) (**H**) *M. wikkiensis* (scale 2) (**I**) *M. tarrissei* (scale 3).

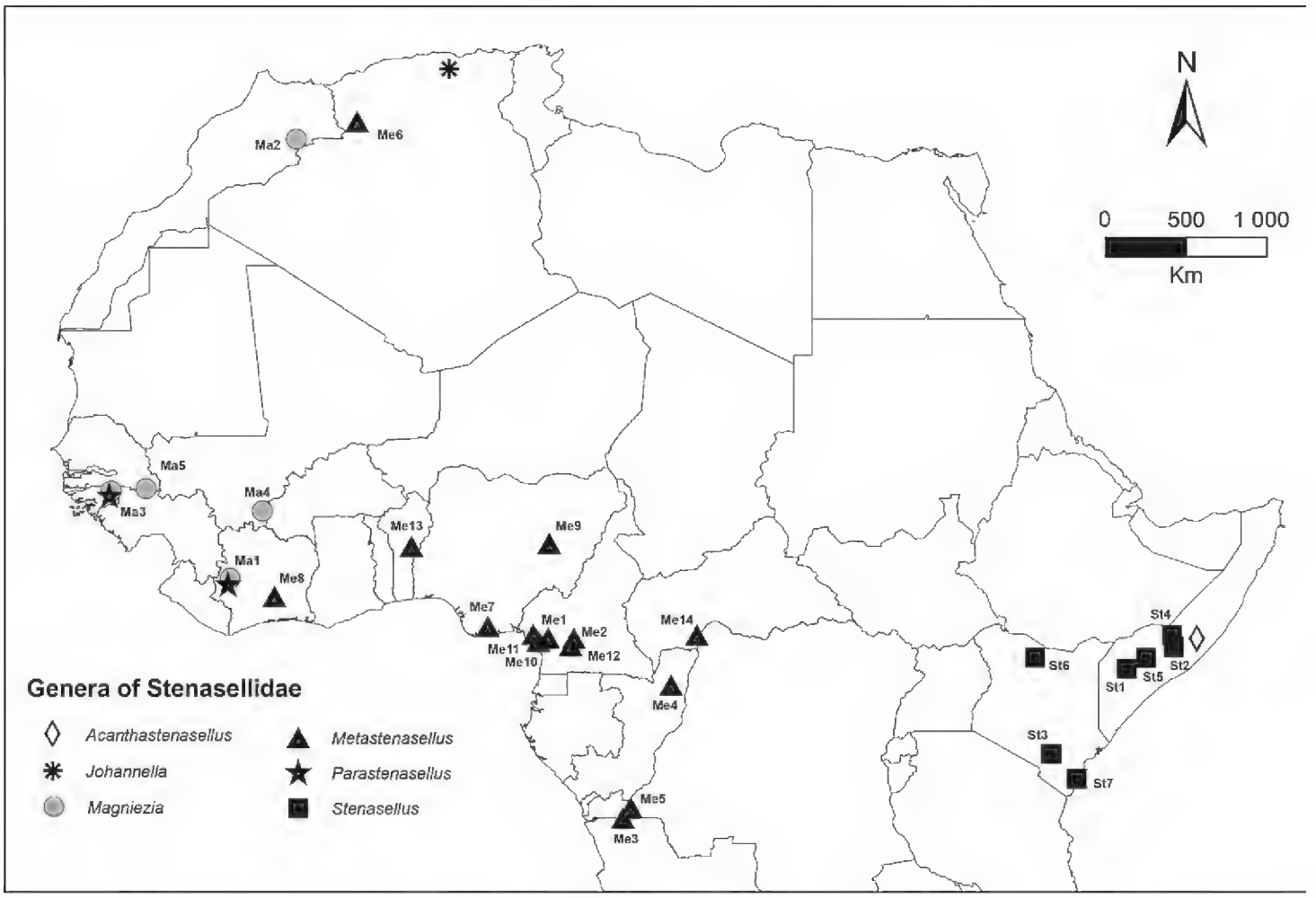

Figure 7. Distribution map of Stenasellidae in Africa: *Acanthastenasellus* (*A. forficuloides*); *Johanella* (*J. purpurea*); *Magniezia* (Ma1: *M. africana*, Ma2: *M. gardei*, Ma3: *M. guinensis*, Ma4: *M. laticarpa*, Ma5: *M. studiosorum*); *Metastenasellus* (Me1: M. boutini, Me2: *M. camerounensis*, Me3: *M. congolensis*, Me4: *M. dartvellei*, Me5: *M. leleupi*, Me6: *M. leysi*, Me7: *M. powelli*, Me8: *M. tarrissei*, Me9: *M. wikkiensis*, Me10: *Metastenasellus* sp1, Me11: *Metastenasellus* sp2, Me12: *Metastenasellus* sp3, Me13: *Metastenasellus* sp. 4, Me14: *Metastenasellus* sp5); *Parastenasellus* (*P. chappuisi*); *Stenasellus* (St1: *S. agiuranicus*, St2: *S. costai*, St3: *S. kenyensis*, St4: *S. migiurtinicus*, St5: *S. pardii*, St6: *S. ruffoi*, St7: *S. simonsi*).

Discussion

Taxonomic position of *Metastenasellus boutini*

The first stenasellid isopods were found for the first time in France in 1896 and later described as *Stenasellus virei* Dolfus, 1897. This first species was followed by successive discoveries in southern and central Europe in the early 1900's (Magniez 1999) together with the first African stenasellid, *Johannella purpurea* in Algeria (Monod 1924). More recent discoveries of new species in western, central, and eastern Africa have modified the taxonomy of the family in-depth, with the description of seven new genera since 1966 and especially the genus *Metastenasellus* (Magniez 1966). Magniez originally defined this genus as displaying well-developed pleonites 1 and 2, dactyli of pereopods 2–7 with one sternal spine, the male protopodite of pleopod 1 without a coupling hook and the male endopodite of pleopod 2 very voluminous and a helicoidal spermatic duct. A few years later, the diagnosis was slightly updated by changing the relative size of pleonites 1 and 2 compared to the length of pereonite 7 (Magniez 1979). In his original diagnosis, pleonites 1 and 2 reached at least 2/3 of pereonite 7. However, the discovery of *M. wikkien-*

sis Lincoln 1972 with reduced pleonites 1 and 2 (50% of pereonite 7) required an update of the diagnosis of the genus: the ratio of the length of pleonites 1 and 2 to the pereonite 7 has to be higher than 50%. Until the discovery of *M. camerounensis* in Cameroon (Zebaze Togouet et al. 2013), the ratio of 50% was restricted to *M. wikkiensis*. Our description of *M. boutini* with a similar ratio provides a third species of the genus with such a ratio and confirms the diagnostic validity of this criterium for the genus *Metastenasellus*.

Despite the geographical proximity of *M. camerounensis* and *M. boutini*, their morphology differs strongly. The two species have a relatively large size among *Metastenasellus* and a similar pleonites/pereonite 7 ratio, but the shape of pleopod 2 strongly differs. In *M. boutini*, the endopodite of pleopod 2 is evolved, with a distal part of the spermatic duct almost fully inside the second article of the endopodite. This specificity is considered as the ultimate evolution of pleopod 2 in the genus *Metastenasellus* (Magniez 1979), whereas the shape of the endopodite of *M. camerounensis* is much more primitive (50% of the spermatic duct is out of the second article) as for *M. leleupi*. By the shape of the pleopod 2, *M. boutini* is closer to *M. wikkiensis* found in Nigeria than to *M. camerounensis*. However, based on other characteristics such as the external lobes on the protopodite of pleopod 2, *M. boutini* is also close to the dwarf species *M. leisi* found in Algeria.

As suggested by previous studies, the second male pleopod exhibits several significant differences among *Metastenasellus* species (Magniez 1991; Zebaze Togouet et al. 2013). Among the many characteristics of pleopod 2, species can be separated into two main groups according to the shape of article 2 of the endopodite. The *Metastenasellus leleupi* group is characterized by a distal part of the spermatic duct clearly protruding out the second article of the endopodite (i.e a primitive characteristics *sensu* Magniez 1979); the group is composed of 5 species (*M. leleupi*, *M. camerounensis*, *M. dartivellei*, *M. powelli*, *M. congolensis*). The second group, the *M. tarrissei* group is composed of more evolved species whose distal part of the spermatic ducts is almost or fully inside the second article of the endopodite (*M. boutini*, *M. tarrissei*, *M. wikkiensis*, and *M. leysi*). However, the differences between these two taxonomic groups are not sufficient to distribute these species into two genera. Firstly, the morphological characteristics of all species fit the diagnosis of the genus for all traits (Magniez 1979), and secondly the difference among species are not dichotomous but follow a continuous gradient from *M. leleupi* to *M. tarrissei*.

Ecology and distribution of Stenasellidae in Africa

The stygobitic family Stenasellidae is widely distributed in southwestern Europe, the Middle East, Asia, and even Central America (Magniez 1999; Lewis and Sawicki 2016). In Africa, stenasellid isopods can colonize a wide range of environmental niches under very different climate conditions ranging from dry Saharan climate to wet equatorial climate (Magniez, 1999). For instance, the family is known to be tolerant to a very wide range of pH values (from 3.4 for *M. boutini* in this study to 8 for *Acanthastenasellus* in Somalia) as well as a wide range of altitudes (from 0 to 1300 m a.s.l. for *M. leysi* in Algeria) and temperatures (more than 34 °C for *S. rufoi* in Kenya). They have large pleopods 3, 4, and 5 (gills) and their red/pink color indicates a high amount of haemo-

lymph which likely enables them to withstand poor oxygen concentrations, even if this point has not been studied (Magniez 1999). However, and despite their wide environmental tolerance, there is no mention of stenasellids in brackish water and their known localities do not exceed a salinity of 3 $g.L^{-1}$for *S. ruffoi* (Messana, 1993).

Stenasellids can also colonize all kinds of groundwaters (karst, interstitial and phreatic waters) (Magniez 1999). The wide "tethyan" distribution of stenasellids suggests the presence of an ancestor at least during the Upper Cretaceous. This hypothesis is well supported by the molecular phylogeny of Asellota (Morvan et al. 2013) showing that stenasellids were already present on Pangaea during the late Paleozoic (≅ 250 MYA), while African and Nearctic stenasellids were geographically separated. The lack of data about the distribution of stenasellids in Africa does not allow us to draw a clear conclusion about their biogeography in Africa.

Key to *Metastenasellus* species

This identification key concerns males of the nine currently known species of the genus *Metastenasellus*: *M. leleupi* (Chappuis, 1951); *M. congolensis* (Chappuis, 1951); *M. dartivellei* (Chappuis, 1952); *M. wikkiensis* Lincoln, 1972; *M. powelli* Magniez, 1979; *M. tarrissei* Magniez, 1979; *M. leysi* Magniez, 1986; *M. camerounensis* Zebaze Togouet, Boulanouar, Njiné & Boutin, 2013, *M. boutini* Pountougnigni, Piscart & Zebaze (present study).

Like the key proposed by Zebaze et al. (2013), our key is largely based on the second male pleopod. However, the intermediate size of *M. boutini* did not allow us to just update the key proposed by Zebaze et al. (2013). As a consequence, the new key was largely rebuilt, as follows:

1 Pleopod 2, distal part of spermatic duct clearly protruding out of the main part of the second article of endopodite (Fig. 6A–E) **2**

– Pleopod 2, distal part of spermatic duct slightly protruding out or fully inside the second article of endopodite (Fig.6 F–I) **6**

2 Pleopod 2, external part of spermatic duct strongly protruding out (≥ 50%) of the second article of endopodite (Fig. 6A–B) **3**

– Pleopod 2, external part of spermatic duct slightly protruding out (≈ 20%) of the second article of endopodite (Fig. 6C–E) 4

3 Pleopod 2, second article of endopodite subrectangular, external part of spermatic duct half the length of second article of endopodite with a row of chitinous teeth along the last 2 whorls, second article of exopodite with 4 marginal and subterminal setae but without a terminal seta; total body length > 10 mm; (Fig. 6B).... .....***M. camerounensis*** **[Yaoundé, Cameroon]**

– Pleopod 2, second article of endopodite conical, external part of spermatic duct of the same length as the second article of endopodite with a row of chitinous teeth only at the apex, second article of exopodite with 2 or 3 setae with a terminal seta at the apex; total body length < 8 mm (Fig. 6A) ....***M. leleupi*** **[Kinshasa Province, Democratic Republic of the Congo]**

4 Pleopod 2, first segment of endopodite clearly delimited, second article of exopodite with 6 to 8 setae (Fig. 6D-E); flagellum of antenna 2 with more than 45 articles..... **5**

– Pleopod 2, first segment of endopodite not clearly delimited, second article of the exopodite with 5 setae (Fig. 6C); flagellum of antenna 2 with no more than 35 articles..... ***M. dartivellei*** **[Équateur province, northwestern Democratic Republic of the Congo]**

5 Pleopod 2 protopodite longer than wide (Fig. 6D); protopodite margin of pleopod 1 straight; flagellum of antenna 1 with less than 10 articles; total body length < 8 mm..... ***M. powelli*** **[Port Harcourt, southeastern Nigeria]**

– Pleopod 2 protopodite longer than wide (Fig. 6E); protopodite margin of pleopod 1 convex or concave; flagellum of antenna 1 with at least 18 articles; total body length > 10 mm ..... ***M. congolensis*** **[Congo Central Province, eastern Democratic Republic of the Congo]**

6 Pleonites 1 and 2 as long as pereonite 7; flagellum of antenna 1 with less than 8 articles; flagellum of antenna 2 with less than 25 articles; total body length ≤ 5 mm..... **7**

– Pleonites 1 and 2 as long as 50% of pereonite 7; flagellum of antenna 1 with 8 articles; flagellum of antenna 2 with more than 25 articles; total body length ≥ 8 mm..... **8**

7 Pleopods 2 exopodite wider than endopodite; exopodite and endopodite not clearly separated (Fig. 6G); flagellum of antenna 1 with 2 articles; flagellum of antenna 2 with 13 articles; total body length ≤ 3.5 mm .... ***M. leysi*** **[Naâma Province, northeastern Algeria]**

– Pleopod 2 endopodite wider than exopodite; exopodite and endopodite well separated (Fig. 6I); flagellum of antenna 1 with 7 articles; flagellum of antenna 2 with 25 articles; total body length ≅ 5 mm..... ..... ***M. tarrissei*** **[Lake District, central Ivory Coast]**

8 Pleopod 2 protopodite with an external lobe, second article without terminal setae (Fig. 6F); uropod never as long as 50% of the pleotelson..... ..... ***M. boutini*** **[Douala, southwestern Cameroon]**

– Pleopods 2 protopodite without an external lobe, second article with a terminal seta (Fig. 6H); uropod twice the length of the pleotelson for the longest males ... . ***M. wikkiensis*** **[Plateau State, northeastern Nigeria]**

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Supplementary material 1

List ot sampling sites with ot without *Metastenasellus boutini* in the area arround the type locality

Authors: Pountougnigni Oumarou Farikou, Piscart Christophe, Sob Nangou Paul Bertrand, Zebaze Togouet Serge Hubert

Data type: occurrences

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